where NA is the numerical aperture on the object side of the objective. The depth of field, D, is related to the depth of focus by the longitudinal magnification of the system, m^2, so that $D = D'/ m^2$ or

$$D = \lambda/(NA)^2$$

For an oil immersion type objective the index of refraction of the oil must be accounted for and the depth of field is n times larger than the above.

High numeric aperture microscope objectives used with some of the implementations of the imaging system 100 are readily available commercially with NA values ranging from 0.5 to 1.4. For visible light imaging, assuming a center wavelength of $\lambda = 0.55$ microns, these NA values translate to tolerable depths of field from as little as 0.4 microns to 4.0 microns. Tolerances for allowable depth of focus other than Rayleigh's criterion may result in an expansion or reduction of this range. For example, a decrease in the modulation transfer function at a particular spatial frequency might be the acceptance criterion for implementation of the imaging system 100.

In some implementations of the imaging system 100 for biological cell imaging in flow, collection lens are microscope objectives of 40X magnification with 0.9 NA and the imaging lens has a focal length of 200mm. Cell objects are nominally 10 microns in diameter and the imaging field of view orthogonal to the flow axis is set to be 30 microns. Detector pixel size is approximately 13 microns. Consequently, the desired lateral separation between unaltered and defocused focal plane images at the detector is 100 pixels or 1.3mm. The lateral separation at the detector is given by $f*\tan\phi$, where f is the focal length of the imaging lens and ϕ is the optical angle of separation. For the 200mm focal length lens the angle of separation is 6.5 milliradians to achieve the 1.3mm lateral separation. Note that this translates to a mechanical angle of 3.25 milliradians for the beam combiner element, since upon reflection the optical angle is twice the mechanical angle of the reflective surface. The depth of field for the 0.9NA objective is 1.03 microns and the required optical power introduced into the defocused optical path is $\pm$ 0.04 diopter, corresponding to a defocus lens focal length of $\pm$ 25 meters. This optical power results in a

separation of the unaltered and defocused object planes by 1 micron, to nearly double the depth of field of the system.

Numerous implementations of the imaging system 100 can be accomplished with a variety of components. In the biological application objects are cells of typically 5 to20 microns in diameter. In other implementations, microscopic objects of interest may have a size range of 1 to 50 microns. High NA microscope objectives are commercially available from 10X/0.5NA to 100X/1.4NA with optical designs optimized for use with imaging lens focal lengths of 165 to 200mm. Typical CCD detector pixel sizes range from 5 to 25 microns. Optical systems employing these components in various embodiments may require optical power in the defocused optical path to range from ± 0.01 to ± 0.1 diopter. Angular separation between the unaltered and defocused optical paths may range from as little as 0.1 degree to 10 degrees. However, those skilled in the art will appreciate that other optical system applications with different imaging requirements can be constructed with custom designed components that may extend these typical parameter ranges.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.